In the United States Patent and Trade Mark Office

Re: Patent Application

Serial Number 10/031,874 of Tanha *et. al.*, Filed November 14, 2002;

Examiner David J. Blanchard

Declaration of Arumugam Muruganandam

I, Arumugam Muruganandam, am employed as Senior Scientist at Dyax Corporation, Cambridge, MA, USA. I have been employed in this capacity since April 2001. Prior to this, I was employed as an Associate Research Officer at the National Research Council of Canada, Ottawa, Canada.

Except where otherwise noted, I have personal knowledge of the statements made herein. This Declaration reflects my personal expert opinion and is not made in the course of my employment by Dyax Corporation, nor should it be considered a statement by or on behalf of my employer.

I have 7 years of experience in the area of recombinant antibody and phage display technologies and consider myself to be an expert in this field. Details of my professional accomplishments are listed in my curriculum vitae which is Exhibit "A" to this Declaration.

I have read the reference of Casterman et al (WO 9404678), and I am familiar with and fully understand its contents. I have also read the disclosure and claims of United States Patent Application Serial Number 10/031,874 of Tanha *et al.*, am familiar with it and fully understand its contents.

The reference of Casterman et al does not provide the critical technical information necessary for the preparation of naïve llama VHH single domain antibody libraries where antibodies with usefulbinding affinity could be obtained. In particular, Casterman et al failed to teach use of a multibvalent display vector such as a phage vector which as a practical matter would be a practical requirement for the preparation of any such single domain polyvalent library.

Moreover, Casterman et al fails to disclose what specific oligonucleotide primer set one should use to prepare such antibody library. The use of the correct sequence specific complimentary oligonucleotide primer set is a necessary step for the production of such antibody library. The description provided by Casterman et al in his reference is a bare and general description of the procedure used in relation to conventional Fab and scFv libraries, without the additional teaching necessary to allow the production of VHH single domain binder libraries with useful binding affinities, including those with a dissociation constant of 5.7 x 10 exp-5 M or lower. In my view, the method disclosed by Casterman failed to direct the choice of a polyvalent display in preparing a naïve library.

If someone skilled in this area read Casterman et al reference and used the generally accepted procedures for the preparation of libraries, that person would have used a phagemid vector and not a phage vector, because that is and was,

standard practice. Any broad references in Casterman et al to the use of a phage display library would be interpreted by one skilled in the art in favour of phagemid vectors because of knowledge in the field which supports the use of phagemid vectors for library production.

Subsequent papers of Casterman's group support this, as they have written since the publication discussed herein that pre-immunization of animals is essential for the generation of high-affinity antibody fragments using his method. In particular, see Ghahroudi *et al.* FEBS Lett. 1997 Sep 15;414(3):521-6.

Phagemid vectors are primarily suitable for use in preparing monovalent display libraries of antibodies having high intrinsic binding affinities, i.e., antibodies generated by immunization of animals.

Phage vectors are necessary for the generation of high affinity antibody fragments from antibodies having an intrinsically low affinity for antibody, as is the case for single domain libraries from a naïve llama. Thus, the use of a phage vector (where multivalent display in phage confer a high apparent affinity on intrinsically low affinity binders), as opposed to a phagemid vector, is a practical necessity to the generation of high-binding affinity antibody fragments from a naïve llama.

Thus, a person skilled in the art who read Casterman et al would (i) have found that Casterman et al failed to teach a means to generate a naïve library; and (ii) would have relied on common general knowledge in the area which teaches away from the use of phage vectors which enable the production of the fragments described and claimed by Tanha *et al.*.

I hereby declare that all statements are made of my own knowledge, are true and that all statements made on information and belief are believed to be true and further that these statements are made with the knowledge that willful, false statements and the like so made are punishable by fine or imprisonment, or both under § 1001 of Title 18 of the United States Code, and that such willful statements may jeopardize the validity of the application or any patent issued therefrom.

1/13/05
Date

A. Murugan Dan
Arumugam Muruganandam

Witness Signature

Witness Name Printed Dan Sexton Date 1/13/05